



Temporal trends of biochemical parameters in brain-dead patients.

KHADEJA BI A ALTAFF

**Department of Biochemistry,
MADRAS MEDICAL COLLEGE AND GOVERNMENT GENERAL HOSPITAL**

Abstract : INTRODUCTION Brain death is the irreversible cessation of brain function. Patho-physiological changes like hemodynamic instability, endocrine disturbances, hypothermia, coagulopathy, persistent hypoxemia and electrolyte imbalance are observed in brain-dead patients. A brain-dead organ donor provides a life-saving opportunity to the recipient. Hence, earliest identification of brain death and effective correction of the above changes is required to optimize the harvest and enhance the survival of graft. With this in mind the study was undertaken to find out the pattern of changes occurring in the serum levels of common analytes. OBJECTIVES 1. To analyze the biochemical parameters namely serum glucose, urea, creatinine, sodium and potassium in patients declared brain-dead in our hospital for a period of 19 months from January 2012 to July 2013. 2. To interpret the temporal rise or fall of serum glucose, urea, creatinine, sodium and potassium over a period of 24 hours following brain death. METHODS 42 patients aged between 20 and 40 years, admitted to our hospital and declared brain-dead

after admission, were selected for the study. The biochemical parameters listed along with the methodology were analysed within the temporal trends, over a 24 hour period. 1. Serum glucose by Glucose-Oxidase-Peroxidase method 2. Serum urea by DiacetylmonoximeThiosemicarbazide method 3. Serum creatinine by modified Jaffes method 4. Serum sodium potassium using ion selective electrode. RESULTS The average age of the study population was 29.34 \pm 6.8 years. Of the 42 patients 39 were males while 3 were females with head injury following a road traffic accident. During the 24-hour study period maximum incidence of hyperglycemia and hypokalemia was observed in the 3 to 6 hours samples. Serum urea, creatinine and sodium were invariably in the normal range. Elevated serum urea and sodium were more commonly observed during the 0 to 2 hour period. CONCLUSION Hyperglycemia, hypokalemia and hypernatremia are more frequently reported in brain-dead patients. Timely identification by frequent sampling and appropriate correction of these factors will improve the quality

of the organs to be transplanted.

Keyword : Biochemistry in brain-dead, hyperglycemia associated with hypokalemia

DEFINITION OF BRAIN DEATH:

Brain death is the cessation of brain function with zero percent recovery. In 1968, Ad Hoc Committee of Harvard Medical School defined brain death as irreversible coma with the patient being totally unresponsive and unresponsive with absence of all cranial reflexes and no spontaneous respiratory efforts during a brief period of disconnection from the ventilator(1). Coma differs from brain death. Coma is a state of unconsciousness where the brain still functions and the individual is capable of breathing without the help of a ventilator. The brain, in fact, may heal. Brain death results from total, irreversible loss of all brain functions, due to severe head injury or illness. The heart beats for a period of time after entering into a state of 'BRAIN DEATH'. This rare phenomenon makes ORGAN DONATION possible. However, in spite of the assistance with a ventilator for breathing, the time between the brain death and the cessation of heart beat, does not exceed 24-48 hours. Hence it is important to decide about organ donation before the heart stops functioning. The number of recipients for organ transplantation continues to increase as a result of an ageing population and expanding indications for transplantation (2). Successful medical management of the organ donor is critical to actualizing the individual or family's intent to donate and maximizing the benefit of the intent.

CAUSES OF BRAIN DEATH

Important causes of brain death include

1. Severe head injury (like road traffic accidents),
- 2 Aneurysmal subarachnoid hemorrhage,
- 3 Intra-cerebral hemorrhage,
- 4 Large ischaemic strokes associated with brain swelling and herniation,
- 5 Hypoxic- ischaemic encephalopathy after prolonged cardiac resuscitation or asphyxia,
- 6 Massive brain edema in patients with fulminant hepatic necrosis.

PATHO-PHYSIOLOGICAL CHANGES OF BRAIN STEM DEATH:

The organ donor presenting with BRAIN DEATH is under a distinct and challenging pathophysiologic condition resulting from multifactorial shock. The time interval between declaration of brain death to organ procurement ranges from 12-48 hours and is related to the time required for executing the various procedures to emphasize the declaration of brain death, consent discussions with the family members, procurement logistics of donor/ organ evaluation, and donor/ recipient matching (3). During this phase, risks for hemodynamic instability and compromise of end-organ function are high. In the Post Anesthesia Care Unit (PACU), patients declared BRAIN-DEAD are monitored using protocols similar to the management of any patient with multifactorial shock. Understanding the physiology of brain death is important in order to treat the donor,

which is as much important as treating a transplant recipient.

In the initial period following a traumatic brain injury, there is a raise in intra cranial pressure (ICP) causing mean arterial pressure to raise and maintain the cerebral perfusion. When ICP continues to increase, cerebral herniation into the brain stem ensues resulting in its ischemia in a rostral to caudal fashion. Initial apnea, bradycardia, hypotension and ultimately, a drop in cardiac output are mediated by vagal (parasympathetic) activation resulting from midbrain ischemia. Brainstem ischemia then progresses towards the pons, where sympathetic stimulation is superimposed on the initial vagal response resulting in tachycardia and hypertension (Cushing's reflex). When Marey's reflex (hypertension induced fall in heart rate) gets superimposed bradycardia and hypertension is noticed.

Further extension into the medulla oblongata occurs, at which point the vagal cardiomotor nucleus becomes ischemic, preventing tonic vagal stimuli. This results in unopposed sympathetic stimulation, called the 'AUTONOMIC STORM'. This phase occurs unheralded and is of variable duration, during which time hyperglycemia, cardiotoxicity and severe vasoconstriction occur compromising end organ perfusion. This is of critical significance in chemosensitive organs like the heart and liver where immediate graft functioning is essential.

Following the autonomic storm, there is a profound reduction in the sympathetic outflow and catecholamine levels decrease to below baseline values. The resting vagal tone is abolished due to the destruction of the nucleus ambiguus. Hypotension is frequently encountered in the subsequent chronic maintenance phase.

Whether the functions of the hypothalamus is preserved, is yet to be established. The level of vasopressin, a hormone produced in the hypothalamus and stored in the posterior pituitary, decreases significantly after brain death (4). However, the occurrence of diabetes insipidus is variable. In one study, many patients did not have diabetes insipidus after brain death (5). Another study found that 24 of 31 patients with brain death had clinical diabetes insipidus (6). It was reported that plasma levels of the thyroid hormones, triiodothyronine (T_3) and thyroxine (T_4) were decreased markedly after brain death (7).

Hypothalamic dysfunction results in the loss of thermoregulation, reflecting with hypothermia. It is exacerbated by the reduced metabolic rate, functional hypothyroidism, and vasodilatation, seen in the donors. Coagulation abnormalities can occur, secondary to the effects of catecholamines on platelet function and the release of plasminogen activator along with thromboplastin in response to damaged brain tissue.

Hyperglycemia observed in brain-dead organ donors can be due to increased levels of catecholamines, reduced insulin secretion and administration of dextrose during maintenance phase. Hyperglycemia results in an osmotic diuresis and electrolyte disturbance. Electrolyte abnormalities observed in brain-dead organ donor include hypernatremia, hypokalemia, hypocalcemia, hypomagnesemia and hypophosphatemia. Elevated blood lactate levels observed in brain-dead patients suggest an occult systemic hypoperfusion state, resulting in anaerobic metabolism (8).

CLINICAL DIAGNOSIS OF BRAIN STEM DEATH:

The three cardinal findings in brain death are

1 Coma or unresponsiveness,

2 Absence of brain stem reflexes and

3 Apnoea. Testing for the same is carried out during bedside clinical examination in the presence of a neurophysician and neurosurgeon. Electroencephalography and conventional angiography are generally accepted as confirmatory tests for declaring a patient brain dead.

Supportive care for the Brain-dead Organ Donor:

After the diagnosis of brain death, the focus of patient care shifts from interventions aimed at saving the donor's life to interventions aimed at maintaining the viability of potentially transplantable organs. The main goal of organ donor management is the maintenance of optimal conditions that will ensure functional, intact and sterile organs. The quality of organs to be recovered is preserved by optimal management of hydration, perfusion, oxygenation, diuresis, temperature control and prevention of infection.

Supportive treatment is started as soon as brain death has been recognized irrespective of the consent. Otherwise there can be a rapid deterioration of initially suitable donors. In a poorly managed donor, the exercise of organ donation yields organs that result in poor graft outcomes. The use of protocols has been shown to increase the number of organs recovered by 71% and decrease the number of donors lost due to instability by 87%. Protocols are helpful in guiding the clinical team by reinforcing the importance of aggressive donor management, reminding staff of general measures and specific drug

therapies and detailing physiological targets. Protocols also help to guide the management of social aspects of organ donation. Common clinical problems encountered in the brain stem dead patients

1. Hypotension

2 Hypothermia

3 Diabetes insipidus

4 Hyperglycemia associated with hypokalemia

5 Pulmonary edema

6 Hypoxia

7 Metabolic acidosis

8 Arrhythmias

9 Disseminated intravascular coagulation

Infections. Despite the use of well-accepted protocols for donor maintenance, high incidence of disorders like hyperglycemia, hypokalemia, hypernatremia, hyper-osmolality, hypophosphatemia, elevated lactate levels are reported (8). Timely identification and appropriate correction of these factors will avoid occult ischemia of organs and consequently improve their quality for transplantation.

METHODOLOGY:

42 patients aged between 20 and 40 years, admitted to our hospital and declared to be presenting with brain death, were selected for the study. Levels of biochemical parameters like serum glucose, urea, creatinine, sodium, potassium were studied for a period of 24 hours from the time of admission of the patients.

Serum glucose was estimated by Glucose oxidase- peroxidase (GOD-POD) method. Glucose is oxidized to gluconic acid and hydrogen peroxide, by GOD. POD converts hydrogen peroxide to nascent oxygen which in turn oxidises colourless chromogen to pink coloured complex and OD is measured at 540nm using spectrophotometer.

Serum Urea was estimated using Diacetyl monoxime- Thiosemicarbazide (DAM-TSC) method. Urea reacts with DAM in the presence of ferric ions to give a pink coloured complex which is measured at 540nm. TSC stabilizes the colour.

Serum creatinine was estimated by kinetic Jaffe's method. Creatinine reacts with alkaline picrate to form a reddish orange coloured creatinine picrate and its absorbance is measured at 490nm using a spectrophotometer at 20 and 80 seconds.

Serum sodium and potassium were measured using ion-selective electrodes. Values of serum biochemical parameters considered significant for classification (Table 1 and 2):

Serum glucose(15) 72-144mg/dL Serum urea (16) 15-40mg/dL Serum creatinine (15) 0.6-1.7mg/dL Serum sodium(15) 130-150 mEq/L Serum potassium(17) 3.5-5.1 mEq/L

RESULTS:

Average age of the study population was 29.34 ± 6.8 years. 94% (39 of 42) of the patients were males, while 6% (3 of 42) were females. In the present study, head injury following road traffic accident was the cause for brain-death. In these patients serum glucose, urea, creatinine, sodium and potassium were analysed over 24 hours period. The samples received during the 24 hours were categorized into 0 to 2, 3 to 6, 7 to 12, 13 to 18, 19 to 24 intervals from the time of brain death. The mean, standard deviation and p value of serum glucose, urea, creatinine, sodium and potassium of the brain-dead patients are presented in table 1.

Table 1 shows the mean, standard deviation and p value for glucose, urea, creatinine, sodium and potassium during the 0 to 2, 3 to 6, 7 to 12, 13 to 18, 19 to 24 hours period. Hyperglycemia was observed in majority of the patients during the study period of 24 hours. The incidence of hyperglycemia was maximum during the period of 3 to 6 hours of declaration of brain death. A gradual decline was observed in the means of serum glucose level from 7 to 24 hours. The means for serum urea and creatinine were found to be marginally elevated during the 0 to 18 hours period. The mean sodium and potassium were in the normal and low normal range respectively during the 24 hour study period. Using ANOVA, it was found that there was a significant difference (p value <0.05) in the values of serum glucose and potassium measured at different intervals during 24 hour period. While there was no significant difference in the values of serum urea, creatinine and sodium measured at different intervals during the study period.

Table 2 shows the percentage of brain-dead patients with high, normal and low values of serum glucose, urea, creatinine, sodium and potassium during the 24 hours period. Hyperglycemia was observed in the majority of patients during the study period of 24 hours. The maximum incidence of hyperglycemia was observed in the 3 to 6 hours samples. During this period highest incidence of hypokalemia was also observed. This finding is in concordance with the fact that, insulin released in response to the high blood glucose has also activated the sodium potassium ATPase pump, causing potassium to enter into the cells and producing hypokalemia (9) observed during this

period. Incidence of hypoglycaemia in the study population was very rare and it was observed in the 7-12 hours

sample. Elevated levels of serum urea and sodium were observed in the majority during the 0-2 hour

Table 1, Biochemical parameters namely serum glucose, urea, creatinine, sodium and potassium of brain-dead patients during 24 hours study period.			
	HOURS	MEAN±SD	P VALUE
Serum glucose (mg/dL)	0-2	253.57±117.85	0.017
	3-6	335.88±107.42	
	7-12	311.43±121.53	
	13-18	287.14±139.34	
	19-24	283.29±137.63	
Serum urea (mg/dL)	0-2	49.88±34.08	0.497
	3-6	48.25±34.71	
	7-12	43.17±31.08	
	13-18	43.47±30.16	
	19-24	38.15±14.71	
Serum creatinine (mg/dL)	0-2	1.88±1.40	0.886
	3-6	1.94±1.49	
	7-12	1.87±1.38	
	13-18	1.86±1.21	
	19-24	1.62±1.02	
Serum sodium (mEq/L)	0-2	147.59±13.03	0.497
	3-6	146.07±12.12	
	7-12	143.97±14.86	
	13-18	146.7±16.74	
	19-24	141.04±16.56	
Serum potassium (mEq/L)	0-2	3.62±0.94	0.0007
	3-6	2.84±0.83	
	7-12	3.52±0.97	
	13-18	4.03±1.29	
	19-24	3.42±1.12	

Table 2, Percentage of brain-dead patients with high, normal and low values of biochemical parameters during the 24 hour period.

GLUCOSE	0-2 hours	3-6 hours	7-12 hours	13-18 hours	19-24 hours	P value
High	78.6%	97.6%	90.5%	76.2%	80.95%	0.02
Normal	21.4%	2.4%	7.1%	23.8%	19.05%	
Low	-	-	2.4%	-	-	
UREA						
High	47.6%	38.9%	34.15%	40%	28.13%	0.38
Normal	52.4%	61.1%	65.85%	60%	71.88%	
Low	-	-	-	-	-	
CREATININE						
High	35.7%	36.1%	39.1%	37.5%	25%	0.94
Normal	64.3%	63.9%	60.9%	62.5%	75%	
Low	-	-	-	-	-	

SODIUM						
High	37.5%	36.7%	32.3%	33.3%	29.2%	0.27
Normal	59.4%	56.7%	54.8%	50%	41.7%	
Low	3.13%	6.7%	12.9%	16.7%	29.2%	
POTASSIUM						
High	-	-	12.5%	16.7%	6.45%	0.038
Normal	68.75%	19.35%	20.83%	46.67%	45.16%	
Low	31.25%	80.65%	66.67%	36.67%	48.39%	

Elevated levels of serum urea and sodium were observed in the majority during the 0-2 hour samples. While serum Creatinine was maximally elevated in the 7-12 hour samples. No incidence of low serum urea or creatinine was observed. Highest incidence of hyponatremia and hyperkalemia was observed in the samples obtained between 19-24hours and 13-18hours, respectively. The descriptive variables in table 2 were analyzed using Friedman's non-parametric test. There was a significant difference in the high, normal, low levels of glucose and potassium with p value 0.02 and 0.038 respectively. No significant difference

was observed for the high, normal, low values for serum urea, creatinine and sodium in the 24 hours.

DISCUSSION

Present study on the biochemical parameters of brain-dead patients reveals that there is considerable variation in the levels of serum glucose, urea, creatinine, sodium and potassium during the first 24 hours of brain death. In the present study majority of patients had hyperglycemia in the overall period of 24 hours. The occurrence of hyperglycemic state after brain

death is undisputed (11), however, different views exist regarding the causes of hyperglycemia. It could be due to the catecholamine storm that occurs in the initial period following brain death (3). It is reported that brain death causes significant pathophysiological alterations in the pancreas, including deterioration of pancreatic microvasculature, inflammation, and histologic damage. Each of these sequel plays a role in disrupting beta cell functioning, ultimately destroying the ability of pancreas to secrete sufficient insulin (12). Another view is that the endocrine pancreatic functions are normal after brain death and hyperglycemia results from tissue-insulin resistance (13).

Treatment with corticosteroids, infusion of inotropic drugs and all supportive drugs via 5% dextrose infusion might result in hyperglycemia during the maintenance phase of brain-dead organ donor. Although the mechanism underlying the disruption of serum glucose regulation remains unclear, it is well established that fluctuations occur (14).

In brain-dead patients the hypokalemia observed could be a consequence of treatment with insulin for hyperglycemia. Treatment with intravenous insulin causes rapid shift of potassium from circulation into the intra cellular compartment, resulting in hypokalemia (9). Thus in the present study hypokalemia and hyperglycemia occur together during the 3 to 6 hour period.

Brain death is reported to results in diabetes insipidus (7). The subsequent volume depletion could be a cause for high serum urea and sodium levels observed in the first 2 hour samples. In those with hyponatremia the cause could have been fluid over correction or Syndrome of Inappropriate secretion of Anti-Diuretic Hormone (SIADH) in brain-dead patients. In a study from Iran on brain-dead patients, hyperglycemia was detected in 87% of the donors. Their frequency of main electrolyte abnormalities were, hyponatremia $Na > 147$ mEq/L (58%), hypokalemia $K < 3.5$ mEq/L (25%), hypocalcemia $Ca < 8.5$ mg/dL

$P < 2.5$ mg/dL (60%) (10). The biochemical parameters recorded in the brain-dead patients in the present study showed similarity with this report.

CONCLUSION

Despite the use of well-accepted protocols for donor maintenance, there is a high incidence of hyperglycemia, hypokalemia, hypernatremia. Present study provides basic information on temporal trends in the levels of serum glucose, urea, creatinine, sodium and potassium of the potential organ donors, which is of great use while monitoring them during the maintenance phase. Timely identification by frequent sampling and appropriate correction of these factors will avoid occult ischemia of the organs and consequently improve their quality for transplantation.

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